

Cold Plasma Sterilization of Open Wounds: Live Rat Model

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ABSTRACT: Atmospheric pressure non-equilibrium (cold) plasmas are already known to be effective sterilization agents. This work further confirms the ability of cold plasma to inactivate pathogenic organisms in a live animal model. The rat wound model used here represents a surgical treatment situation whereby the wound is open and the bleeding is controlled. High concentration of *Staphylococcus aureus* is placed onto the wound area and allowed to incubate for four hours prior to cold plasma treatment. We show a 3-log reduction in pathogen load on the wound following a one minute treatment.

KEY WORDS: cold plasma, non-thermal plasma, dielectric barrier discharge, floating electrode, sterilization, animal model, rat, plasma medicine

I. INTRODUCTION

Of the vast number of plasma applications in industry, uses of plasmas in biology and medicine have recently gained significant interest¹. One of the most widely discussed potential applications is plasma treatment of wounds for the purpose of reducing microbial load and enhancing healing processes²⁻⁶. Two major types of plasma treatment have been discussed in connection with wound disinfection. One is indirect treatment using gas flow through plasma which brings mostly neutral active species in contact with living tissue. The other is direct application of non-thermal plasma to living tissues³. Indirect treatment permits to decouple plasma system design from constraints related to applying the treatment to living tissues. For example, it permits to employ thermal plasma for generation of active species including substantial quantities of NO⁷⁻¹⁰. Indirect plasma treatment of skin and wounds has been investigated over the last several years demonstrating a significant effect⁴. On the other hand, applying non-thermal plasma directly to living tissue makes it possible to employ charges and short living neutral species^{11, 12}. It has been demonstrated that bacterial inactivation on surfaces of agar and even skin can be achieved significantly faster with direct exposure to non-thermal plasma such as the Dielectric Barrier Discharge (DBD)³. No significant toxicity was observed during relatively low power and duration plasma exposure which is typically sufficient to achieve significant reduction in the bacterial load^{4, 8, 12, 13}. However, effects of direct DBD plasma treatment on bacterial inactivation in real wounds have not been reported so far.

In this paper we report on a study where open wounds contaminated with bacteria are treated with Floating Electrode –DBD plasma directly. Rat is employed as the animal model. Although bleeding is stopped and most of the excess wound fluid is removed prior to plasma application, the wound remains moist. The results demonstrate that over 3-log reduction (over 1000 times) of bacteria occur following only 1 min of DBD plasma treatment without any visible tissue damage.

II. MATERIALS AND METHODS

The particular form of cold plasma used in this manuscript was used by authors in the previous in-vitro sterilization and blood coagulation studies¹² and in the in-vivo for intact skin toxicity and sterilization⁴. The wound model was developed specifically for this study to simulate an open wound.

A. Floating electrode dielectric barrier discharge

In this study we have used a floating electrode dielectric barrier discharge (FE-DBD) generated between an insulated high voltage electrode and the sample (FE) undergoing treatment. Half millimeter thick polished clear fused quartz (Technical Glass Products, Painesville, OH), was used as an insulating dielectric barrier. The setup and high voltage electrode schematic is shown in Figure 1. The discharge was generated by applying high voltage pulses with the following characteristics: 20 kV (p-p), 1.6 μ s pulse duration, 1 kHz frequency. The average power density for the active area of the high voltage electrode was kept at the level of approximately 0.5 W/cm², i.e. 0.74 Watt for 15 mm electrode diameter. The electrode was specifically designed to fit into the Polycarbonate holder as is shown in Figure 1 and a set of spacers of 0.5 mm thickness was provided to the operator to allow for controlled application of plasma for every wound.

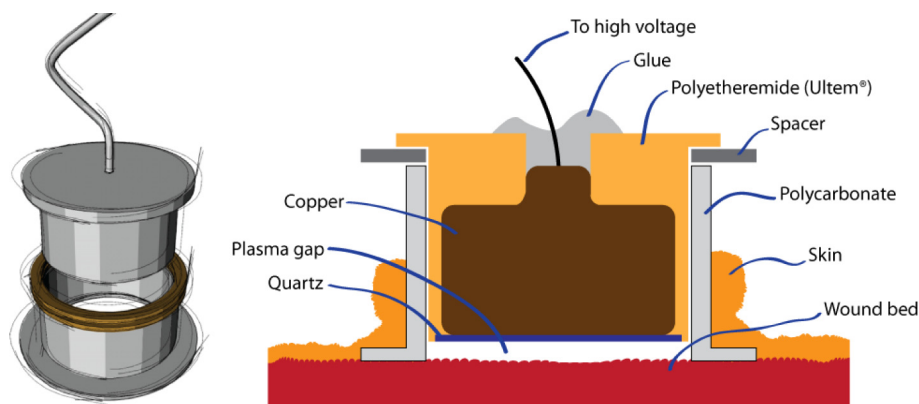


FIGURE 1. Schematic of the FE-DBD plasma electrode that fits into the plastic ring sutured to the wound.

All procedures were performed in compliance with the animal welfare and protection act following the Drexel University's Institutional Animal Care and Use Committee (IACUC) approval.

B. Animal model

Thirty hairless Sprague-Dawley rats, weighing approximately 250 g, arrived and acclimated for 3-5 days in the facility. Animals were transferred from the vivarium to the approved surgical suite. Animals were continuously anesthetized with inhalational gas anesthetic, Isoflurane (Vedco, St. Joseph, MO) at 2-3% induction plus oxygen 2L/min then 1.5-2% maintenance administered via a face mask for the appropriate length of time and placed in the supine position. Animals received analgesia (Meloxicam, Boehringer-Ingelheim, Germany) at 1 mg/kg, subcutaneous, Bupivacaine 25% (Hospira, Lake Forest, IL) 0.5 ml/site intradermally and Normosol R (Baxter, Deerfield, IL) crystalloid parenteral fluid therapy at 10ml/kg subcutaneous bolus at the onset of anesthesia but prior to surgery. The ventral surface of the rat was prepared in sterile surgical fashion: the surgical area was gently washed and sanitized with chlorhexidine scrub solution; the area underwent an initial contact scrub of 5 minutes, rinsed with normal saline solution and repeated second time; a preparatory solution of diluted chlorhexidine with isopropyl alcohol (1:8) was lightly sprayed onto the surgical area. Approximately 2 cm incisions along the right and left side of the thoracic region were inflicted and plastic rings were inserted into the subcutaneous layer of the skin (ring size was equivalent to the size of the plasma probe) and restricted the bacteria to the affected area only, Figure 2). The skin was sutured with 3-0 nylon around the ring in a continuous suture pattern. Twenty μ l drops of PBS solution containing *S. aureus* at concentration of 10^5 or 10^6 ml⁻¹ were applied to the wounds and covered and secured with sterile Tegaderm dressing (3M, St. Paul, MN). Animals were recovered from anesthesia in a pediatric warming incubator and bacteria incubated for four hours. Upon completion of bacterial incubation, animals were re-anesthetized, wounds were opened and treated with DBD or untreated controls. After treatment, wounds were swabbed using sterile technique and transferred into PBS for further dilution and plating and plated directly onto BHI agar. Bacteria inactivation efficiency by DBD plasma treatment was analyzed after 14 hours of incubation on BHI agar. Figure 2 shows the sequence of steps taken during the animal procedure.

All data is reported with 95% confidence interval ($p < 0.05$) and the number of samples in each experiment is identified.

III. RESULTS AND DISCUSSION

The results of viable *Staphylococcus aureus* inactivation in living tissue of experimental wound using FE-DBD plasma for initial concentration of bacteria of 10^5 or 10^6 cells per ml of PBS are shown in Figure 3. We observed a 3-log reduction of bacteria after a one minute exposure to plasma, and the results are similar for the both initial concentrations of bacteria.

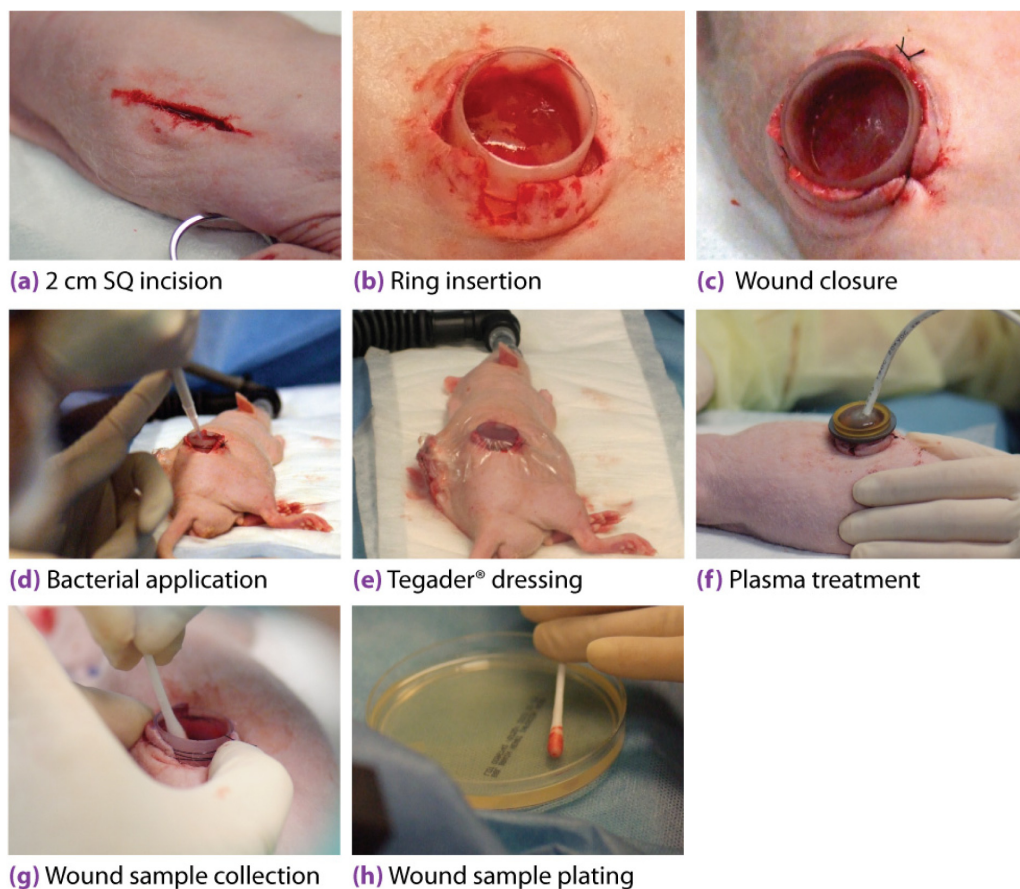


FIGURE 2. Steps taken during the animal procedure: (a) a cut is made; (b) plastic ring is inserted into the skin and (c) sutured in place; (d) wound area is inoculated with known concentration of *S. aureus* and (e) covered with sterile dressing; 4 hours following inoculation the sterile covering is removed and (f) wound area is treated with plasma; after the treatment (g) the wound area is thoroughly swabbed and (h) plated for bacterial counts.

Interestingly, the inactivation efficiency of plasma treatment in the wound is about an order of magnitude less than that for the agar surface^{4, 12}. Here we propose two possible explanations of this effect. First, it is necessary to mention that even under assumption of discharge uniformity (i.e. discharge was covering the whole surface of the electrode, which in present setup was impossible to control), direct plasma exposure has provided only at ~75% of the wound surface due to presence of dielectric enclosure around central copper. Therefore, it is possible that the remaining ~25% of bacteria, which were relatively uniformly distributed over the wound, were treated indirectly. Indirect plasma exposure, i.e. when there is no direct exposure of the treated bacteria to charged species, was previously shown to be about an order of magnitude less effective

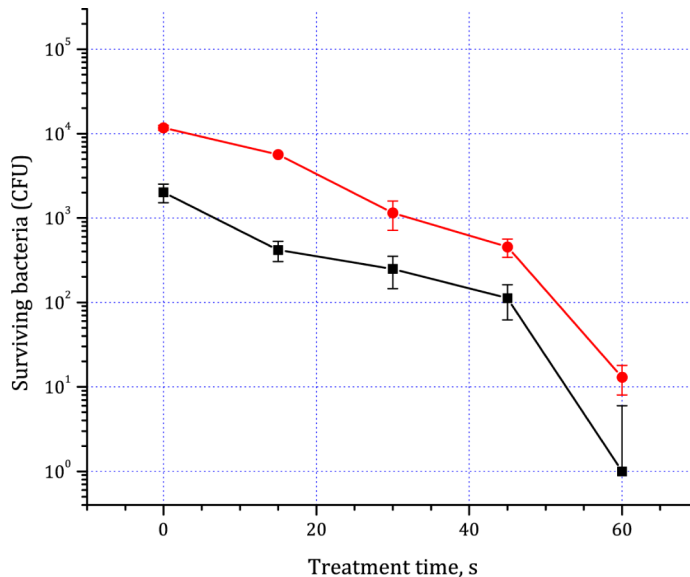


FIGURE 3. Results of wound sterilization with 103 and 104 starting bacterial load.

in bacteria inactivation on agar than direct treatment³. The second hypothesis is related to the presence of wound liquids – compared to almost dry, covered with a minute amount of water, “moist” surface of agar. Again, as we have previously shown, liquid water plays as a “protective” layer, preventing direct delivery of charges to the bacteria surface, and significantly dilutes the inactivation effect of active neutral particles^{3, 11}. At the same time, this wound liquid, which contains high amount of organic molecules, and wound itself may also react with plasma produced reactive species, resulting in decreased activity of plasma treated medium.

IV. CONCLUSION

While we have previously shown effective inactivation of pathogenic organisms in many regimes of plasma and on various surfaces (see^{4, 11} for examples) we have not previously addressed sterilization of living open wound tissue such as would be present during surgery. In this manuscript we show that sterilization is possible and is quite effective of an open wound area of bacteria that was allowed to “settle” in the wound for 4 hours. While the treatment appears to not have any adverse or toxic effect on the wound tissue (as was previously the case with intact skin¹²) an open question remains of the effect of plasma on the wound itself and on the wound healing rate. Since plasma was previously shown in-vitro to generate reactive oxygen species (ROS) which were shown to promote cell proliferation¹³ and in this work was confirmed in-vivo to produce bacterial inactivation similar to in-vitro studies we may assume that wound healing time will be reduced following the treatment. This, however, remains to be verified and is the subject of our follow-on studies.

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